Organic Agriculture and Nitrous Oxide Emissions at Sub-Zero Soil Temperatures

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ABSTRACT

In the Red River Valley of the upper midwestern United States, soil temperatures often remain below freezing during winter and N_2O emissions from frozen cropland soils is assumed to be negligible. This study was conducted to determine the strength of N_2O emissions and denitrification when soil temperatures were below zero for a manure-amended, certified organic field (T2O) compared with an unamended, conventionally managed field (T2C). Before manure application, both fields were similar with respect to autotrophic and heterotrophic N_2O production and N_2O flux at the soil surface (0.15 ± 0.05 mg N_2O–N m^{-2} d^{-1} for T2O and 0.12 ± 0.06 mg N_2O–N m^{-2} d^{-1} for T2C). After application of pelletized, dehydrated manure, average daily flux (based on time-integrated fluxes from 20 November to 8 April), was 1.19 ± 0.34 mg N_2O–N m^{-2} d^{-1} for T2O and 0.47 ± 0.37 mg N_2O–N m^{-2} d^{-1} for T2C. Denitrification for intact cores measured in the laboratory at $-2.5°C$ was greater for organically managed soils, although only marginally significant ($p < 0.1$). Cumulative emissions for all winter measurements (from 16 November to 8 April) averaged 1.63 kg N_2O–N ha^{-1} for T2O and 0.64 kg N_2O–N ha^{-1} for T2C. Biological N_2O production was evident at sub-zero soil temperatures, with winter emissions exceeding those measured in late summer. Late autumn manure application enhanced cumulative N_2O–N emissions by 0.9 kg ha^{-1}.

O RGANIC agriculture currently represents 0.5 million hectares of U.S. cropland and interest in conversion from synthetic to organic-based fertilizer amendments is expected to rise with the cost of fossil fuel-based fertilizers. Agricultural fertilization worldwide contributes 6.2 Tg N_2O–N yr^{-1} to a total global source strength of 17.7 Tg N_2O–N yr^{-1} (Kroeze et al., 1999), and considerable effort has been devoted toward identifying how fertilizers influence the net flux of N_2O (Mosier et al., 1997; Mummey et al., 1998; Flessa et al., 2002; Scott et al., 2002). Nitrous oxide is a concern due to its global warming potential (Robertson et al., 2000), yet data are lacking for winter N_2O emissions in agriculturally productive areas of the upper midwestern United States, where sub-zero soil temperatures persist over a prolonged winter.

Agricultural fertilization influences N_2O fluxes, but the response varies with soil properties, fertilizer type, application timing, and rate (Breitenbeck and Bremmer, 1986; Parton et al., 1996; Mosier et al., 1998; Bouwman et al., 2002). Intensive laboratory studies indicate significant response in N_2O flux when soils are amended with synthetic fertilizers, composts, and animal manures (Breitenbeck and Bremmer, 1986; Paul et al., 1993; Akiyama et al., 2004). The effects of organic fertilizer on N_2O fluxes are complicated by the wide variety of manure

used (Akiyama and Tsuruta, 2003) and by gaps in the knowledge of biological N_2O production at sub-zero soil temperatures (Rover et al., 1998). Soil temperatures in the Red River Valley (RRV) typically remain below zero (to 1-m soil depth) between December and March. Consequently, manures are commonly applied to fields in the autumn before soil freezing with the expectation that nutrients are not transformed in frozen soil and are therefore available to crops the following spring. Conventional synthetic fertilizers, such as urea, are applied more often in the spring, just before planting. The effect of autumn-applied manure on winter N_2O fluxes for agricultural soils that remain below 0°C is largely unknown. The goals of this study were to determine if autumn manure amendment would influence N_2O flux and if denitrification contributed to N_2O flux at sub-zero soil temperatures in RRV (47°01′ N; 96°36′ W) production agriculture systems.

MATERIALS AND METHODS

Field Sites

Two 30-ha crop fields were selected: one managed conventionally for >50 yr (T2C) and one managed organically for 5 yr (T2O) in Clay County, Minnesota (Fig. 1). Before conversion to organic production, nutrient amendment and pest control practices were similar. An uncultivated site located in a tree-row [*Populus* (L.)] was selected as background reference (Fig. 1). This reference site is densely covered by grasses [*Bromus tectorum* (L.) and *Elymus repens* (L.)], and has remained undisturbed for over 30 yr. Soils are classified as silt loams, specifically Colvin (fine-silty, mixed, superactive, frigid Typic Calciaquoll) for the organic and reference sites and Bearden (fine-silty, mixed, superactive, frigid Aeric Calciaquoll) for the conventional site (Soil Survey Staff, 2006). Agronomic inputs (Table 1) represent actual product applications required to produce high yields in a production environment. The organic management treatment began in 1998, when landowners converted management practices to meet USDA-certified organic standards.

Both conventionally and organically managed fields were planted to soybean [*Glycine max* (L.)] in May 2004. Before initiation of experiments in July 2004, fertilizer had not been applied to either field for over 1 yr. Chemical analysis for dehydrated manure product applied in the autumn of 2004 is given in Table 2. Amendments applied previously (Table 1), from1999 through 2004, were calculated by multiplying the amount applied (kg ha^{-1}) for each element by the concentration (g kg^{-1}) reported by fertilizer supplier. Sub-plots (8-ha in area) were delineated at least 50 m from field borders with a 2002 ortho-rectified, high resolution satellite imagery (4 m^2 spatial resolution). Ten points were selected at random within each sub-plot using ArcMap (ESRI, Redwood, CA) randomization procedure for soil and gas flux sampling.

Nearly continuous soil temperature (6 to 8 cm depth) data were recorded between 28 June 2004 and 23 Mar. 2005 at a site located 30 km north of the study site in Polk County, MN

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Abbreviations: POM, particulate organic matter; RRV, Red River Valley; WFPS, water-filled pore space.
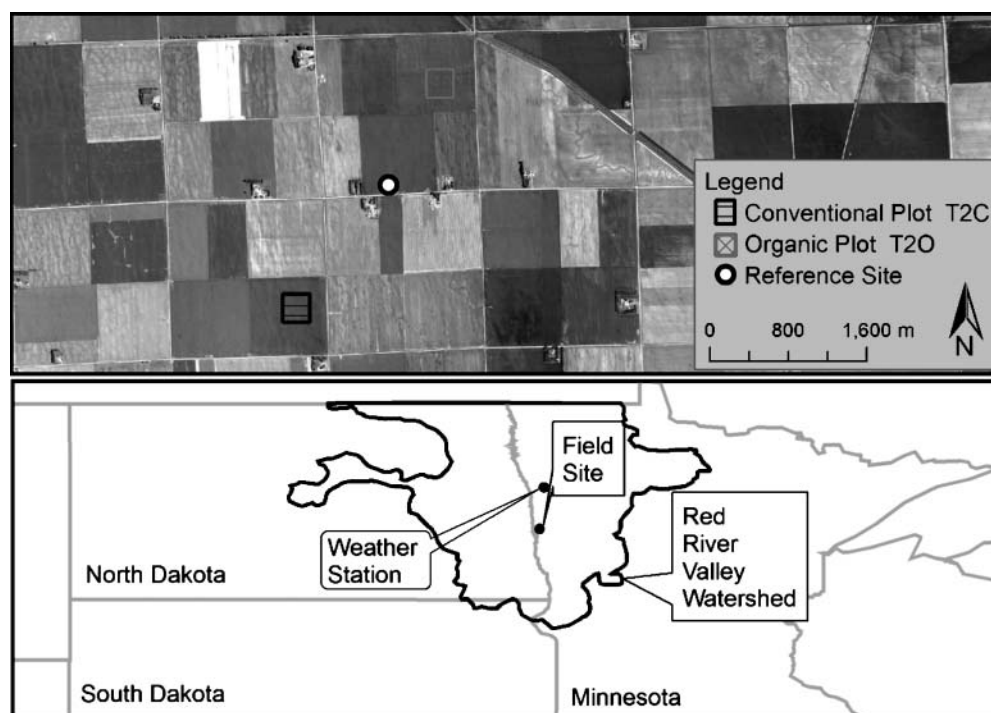
Fig. 1. Overall map of the field site area (47°01′ N; 96°36′ W), located in Clay County, MN where soil and gas flux sample were collected in the Red River Valley (RRV) agricultural region. Weather station location is also shown, from which continuous soil temperature data were collected. Above the regional map is an aerial photograph (2004) indicating proximity of organic field (T2O), conventional field (T2C), and reference site to each other.

(96°40′ W; 47°47′ N). This instrument was equipped with two type E thermocouple probes (model TCAV), which were placed at 6- and 8-cm soil depths (Campbell Scientific). Average soil temperature from 6 to 8 cm were recorded every 0.5 h. Data gaps in late December and January resulted from battery power failure, when daily average air temperature ranged between −4 and −31°C, with an overall average of −18°C during this time period (North Dakota Agricultural Weather Network, 2000).

Soil Properties

Before the autumn 2004 organic manure application, soils were collected to partition sources of N_2O production and to evaluate field-pair suitability. Ten cores (5-cm diam. × 15-cm depth) were collected with a hammer auger from each management treatment and two cores were collected from the uncultivated reference site in July 2004. Cores were kept in cold storage (4°C) until experiments commenced the following day. Samples were obtained at low soil moisture (23% WFPS) to facilitate diffusion of C_2H_2 into the soil pore spaces, as required for selective inhibition of autotrophic nitrification from denitrification and heterotrophic nitrification (Klemedtsson et al., 1988). Field-moist soils were weighed, sieved, homogenized, and analyzed at 20°C for N_2O to determine the relative source strength of autotrophic nitrification vs. heterotrophic processes to N_2O production using sequential application of H_2SO_4–scrubbed C_2H_2. Briefly, N_2O production was first measured (a) from soils under ambient headspace, then (b) with

Table 1. Management history for organic and conventional crop fields. All producer inputs are listed, with the exception of chemical pest control agents (applied on conventional only).

Management	Year	Crop	Tillage	Fertilizer	N	P	K	C
					Management inputs			
					Mg ha^{-1}			
Organic			15 cm chisel	applied in autumn				
	1999	soybean		none	0	0	0	0
	2000	wheat		manure, dehydrated	0.045	0.045	0.056	0.560
	2001	fallow		none	0	0	0	0
	2002	soybean		none	0	0	0	0
	2003	wheat		manure, fresh	0.134	0.162	0.143	1.300
	2004	soybean		none	0	0	0	0
	2005	fallow		manure, dehydrated	0.068	0.050	0.042	0.646
Conventional			15 cm twisted shank	applied in spring				
	1999	sugarbeet		urea	0.020	0.052	0	0
	2000	wheat		urea+potash	0.179	0.067	0.067	0
	2001	soybean		none	0	0	0	0
	2002	sugarbeet		urea	0.020	0.052	0	0
	2003	wheat		urea+potash	0.179	0.067	0.067	0
	2004	soybean		none	0	0	0	0
	2005	sugarbeet		none	0	0	0	0

Table 2. Chemical composition of organic-certified, dehydrated, partially-composted, pelletized manure purchased from Creekwood Farms, Lake Mills, WI.

Pelletized manure chemical composition	Concentration
	$g\ kg^{-1}$
Carbon	478.52
Water	290.96
Nitrogen	50.40
Phosphorous	36.94
Potassium	30.83
Calcium	99.74
Magnesium	6.81
Sodium	3.76
Boron	0.06
Iron	1.01
Manganese	0.40
Copper	0.12
Zinc	0.45

headspace amended with 10 Pa C_2H_2, then (c) with headspace amended with 10 K Pa C_2H_2, to determine the relative contribution of autotrophic nitrification to total N_2O production (Phillips et al., 2000) and total N emitted from denitrification (Paul and Beauchamp, 1989). Autotrophic nitrification is calculated by taking the difference between total N_2O produced (ambient conditions) and N_2O produced by denitrification and heterotrophic nitrification (under a 10 Pa C_2H_2 headspace). Total denitrified N is determined by incubating soils under a 10 K Pa C_2H_2 headspace, which blocks the reduction of N_2O to N_2.

Before gas sampling, C_2H_2–amended soils were incubated for 4 h and equilibrated with the ambient atmosphere. Headspace pressure was equalized during sampling by injecting an equal volume of N_2 into vessel headspace. Sample aliquots were immediately injected into 15-mL exetainers (Labco Unlimited, Buckinghamshire, UK) and analyzed for N_2O with a Varian Model 3800 Gas Chromatograph and Combi-Pal auto-sampler. In this system, sample is auto-injected into a 1-mL sample loop, then loaded onto columns and routed through a ^{63}Ni electron-capture detector (ultra-pure 95% Argon/5% CH_4 carrier gas) detector. The gas chromatograph was calibrated with a commercial blend of CO_2, CH_4, and N_2O balanced in N_2 (Scott Specialty Gases) following verification of stated concentrations with standards from the National Institute of Standards and Technology. The precision of analysis expressed as a coefficient of variation for 10 replicate injections of 360 and 500 nL N_2O–N L^{-1} standards was <1.5%. The minimum detectable concentration change was ± 4 nL L^{-1}; values within 4 nL L^{-1} were not different from each other. Gas samples were stored <8 h before analysis, and tests showed no change in N_2O concentration during storage. The mass of N_2O–N produced was calculated after accounting for soil mass, the mass of N_2O in the aqueous phase, and headspace dilution with N_2. Vessels were sampled four times over a 0.75-hr time course and rates were calculated from the time linear change in headspace N_2O concentration. Incubation data were analyzed separately with a mixed analysis of variance (Littell et al., 1996) to test for differences between organic and conventional soils for N_2O–N emissions, autotrophic nitrification, and total denitrified N (N_2O + N_2).

Cores were analyzed for total N and total C by dry combustion (Carlo Erba NA 1500 Elemental Analyzer). Soil moisture was measured gravimetrically (oven-dried at 105°C). Soil particle density was measured pycnometrically, texture was assessed hydrometrically, and bulk density computed as the quotient of oven-dried mass divided by field volume. Percentage water-filled pore space (%WFPS) was calculated as the ratio of volumetric soil water content to total soil porosity. Soil pH was measured potentiometrically on 1:2 soil/deionized

water slurries equilibrated for 24 h. Ground soil was used to measure inorganic C on soils after application of dilute hydrochloric acid stabilized with $FeCl_2$ by measuring the amount of CO_2 produced by gas chromatography (Loeppert and Suarez, 1996). Soil organic carbon was calculated as the difference between total C and inorganic C. Particulate organic matter (POM) was estimated using the C content of material retained on a 0.053-mm sieve (Gregorich and Ellert, 1993). Soil characteristics were normalized by soil weight, due to differences in bulk density, and analyzed separately with a mixed analysis of variance to determine if soil variables (pH, bulk density, organic carbon, nitrogen, inorganic carbon, % sand, % silt, % clay, % POM, and soil C/N ratio) were similar for conventional and organic fields. For the soil incubation experiments, differences in %WFPS were also tested similarly.

Organic Amendment Application and Core Sampling

On 20 Nov. 2004, the 10 organic field sites were amended with 67.5 kg N ha^{-1} of a pelletized, dehydrated manure product (Creekwood Farms, Lake Mills, WI) and homogenized into the top 5 cm of soil. Amendment was applied to only 1 m^2 since the field was too wet for heavy equipment. The landowner assisted with amendment application to ensure manure was worked into the soil to resemble typical homogenization following disking. Manure product chemical analyses are given in Table 2. Soil temperatures below freezing were recorded 1 wk following manure application, 17 d after manure application. Five intact soil cores (5-cm diam. × 15-cm depth) were extracted from manure-amended sites in the organic field, five from the unamended conventional field, and one from the uncultivated reference site. Cores were kept in cold storage (−4°C) and remained below freezing for the duration of the experiment. Air temperature was −5.1°C and soil temperature (13-cm depth) was −2.0°C at the time of core collection.

To simulate field conditions and minimize soil disturbance, cores were analyzed the following day and kept intact as they were placed inside a 1-L glass Mason jar equipped with a septum for gas sampling. A laboratory-grade, temperature-controlled (± 0.5°C) freezer (Scientemp, Adrian, MI) was used to maintain cores at field temperature in the laboratory. Samples remained vented to the atmosphere except when performing time-course measurements. Cores were allowed to equilibrate at −2.5°C for 4 h before each experiment. Included in the analysis were two empty jars, or blanks, which were treated as samples throughout the experiment. Before sampling, the freezer was opened briefly and jar covers removed to introduce fresh atmosphere into the headspace for 1 h. Jars were then capped while in the freezer, and a 15-mL headspace sample was drawn and replaced with an equal volume of N_2 to maintain constant pressure. The headspace was sampled every 0.25 h to complete a 0.75-hr time course and analyzed as above. Denitrification was also measured on the same soil cores at −2.5°C using the acetylene inhibition technique (Paul and Beauchamp, 1989), and denitrified N (N_2O + N_2) measured following the protocol described above. Following incubations, percentage water-filled pore space was determined for each core. Effects of management treatment on denitrification were tested with the mixed analysis of variance described previously.

Surface Flux Field Samples

Gas fluxes within each management treatment were repeatedly measured using the static chamber method (Whalen and Reeburgh, 1992). Polyvinyl chloride collars (20-cm diam. × 11-cm height) were deployed in the soil 10 d before the first

flux measurement. Polyvinyl chloride covers fitted with butyl O-rings were placed onto the soil collars during each gas flux measurement. Covers included a capillary bleed to equalize pressure and an O-seal fitting and septa for syringe sampling. Samples of the headspace gas were withdrawn from each chamber at 0.25-h intervals during a 0.75-h time course. Air temperature near the soil surface and soil temperature at 13-cm depth were measured in conjunction with gas flux measurements for each date and field with a Type K temperature probe (Cole-Parmer Instrument, Vernon Hills, IL). Soil moisture content was determined at each point in time until 6 December for the 0- to 15-cm depth zone.

Fluxes were measured only four times between August and September 2004 before organic amendment, and time-integrated, daily average values by treatment are reported. Winter field fluxes were measured between 16 November and 8 April and time-integrated, daily average values by treatment are reported. Post-amendment winter fluxes were also analyzed statistically with a mixed, repeated measures analysis of variance to test for effects of amendment on N_2O flux with soil temperature as a covariate. Cumulative N_2O flux by chamber for each treatment were calculated (Phillips et al., 2000), and daily average fluxes by treatment reported. A test for significant differences between treatments for cumulative flux was performed as described previously with the mixed analysis of variance.

RESULTS AND DISCUSSION

Field data collected before the organic amendment indicate soil properties are similar between conventional and organic fields with respect to soil texture, pH, bulk density, soil organic C, soil inorganic C, soil N, and POM (Table 3). Although the organic field received 1.86 Mg ha^{-1} of organic C inputs over the previous 5 yr (Table 1), evidence of greater organic carbon or greater POM in the top 15-cm of soil was not found. Average N_2O–N fluxes collected from soybean fields on 4 August, 24 August, 31 August, and 30 September ranged from 0.05 to 0.34 mg m^{-2} d^{-1}, with an overall average ($\pm$ SD) of 0.12 $\pm$ 0.12 for T2C and 0.16 $\pm$ 0.15 for T2O. Moreover, N_2O production and the relative contribution of N_2O from autotrophic nitrification and heterotrophic processes were also similar (Fig. 2). There was no significant difference in %WFPS among soils, with an average for all soils of 23 $\pm$ 3.3%. At this %WFPS, autotrophic nitrification accounted for approximately 40% of the total N_2O produced. Bateman and Baggs (2005) found approximately 50% of the N_2O–N produced in arable soils occurred during nitrification at 20% WFPS, while the remaining 50% occurred during denitrification. Results here do not differentiate denitrification from heterotrophic nitrification; however, heterotrophic nitrification in arable soils at low %WFPS has not been reported to date. Total denitrified N (N_2O + N_2) was, on average, <10% greater than the rate of N_2O–N produced during denitrification and/or heterotrophic nitrification. If, as in Bateman and Baggs (2005), N_2O produced after incubation with 10 Pa C_2H_2 occurred during denitrification, then microbial reduction of N_2O to N_2 for soils incubated at 23% WFPS was incomplete (Davidson, 1991) and aerobic denitrification likely (Patureau et al., 2000; Bateman and Baggs, 2005). In summary, pre-amendment N_2O emissions and soil core data (field fluxes under soybean and N_2O source partitioning) suggest physical and microbiological controls on N_2O emissions were similar before 20 November organic amendment application.

In the RRV, uncultivated sites are rare and limited to small areas; nevertheless, uncultivated reference site data were collected to provide background information. Reference site bulk density and total soil N was lower than for organic and conventional soils under soybean (Table 3), likely due to graminoid roots and lack of direct N inputs. Daily average flux ($\pm$ standard error of the mean [SEM]) for the reference site between 4 Aug. and 30 Sept. 2004 was 0.04 $\pm$ 0.02 mg N_2O–N m^{-2} d^{-1}, while daily average flux for T2C was 0.12 $\pm$ 0.06 mg N_2O–N m^{-2} d^{-1} and 0.15 $\pm$ 0.05 mg N_2O–N m^{-2} d^{-1} for T2O. Winter surface fluxes between December and April were not collected due to issues with access. Reference soil N_2O flux measured in the laboratory was 22 $\pm$ 1.2 ng N_2O–N g dw^{-1} d^{-1} (dw = dry weight), whereas cultivated soil N_2O flux measured in the laboratory was <15 $\pm$ 4.4 ng N_2O–N g dw^{-1} d^{-1} (Fig. 2). There is no clear reason why ambient N_2O flux for reference soil measured in the laboratory exceeded fluxes for cultivated soils, although sieving uncultivated soil physically disturbs the soil matrix, which has been associated with enhanced N_2O emissions (Venterea et al., 2005).

Biological denitrification occurred for both conventionally and organically managed soils at temperatures below freezing in the laboratory. Average N_2O emission for soils amended with dehydrated manure at −2.5°C (Fig. 3) was 16.30 ng N_2O–N g dw^{-1} d^{-1}. Average emission for unamended soil cores was 5.63 ng N_2O–N g dw^{-1} d^{-1}. Nitrous oxide emission for soil with no known history of cultivation or fertilization was 0.24 ng N_2O–N g dw^{-1} d^{-1}. When calculated as fluxes of N_2O–N m^{-2} d^{-1} (using jar volume and soil core surface area) average emissions for T2O was 1.51 mg m^{-2} d^{-1}, 0.41 mg m^{-2} d^{-1} for T2C, and 0.22 mg m^{-2} d^{-1} for reference. Total denitrification (N_2O + N_2) for T2O soil cores averaged 19.90 ng (N_2O–N + N_2) g dw^{-1} d^{-1}, while average denitrification for T2C soil cores was 1.68 ng (N_2O–N + N_2) g dw^{-1} d^{-1} (Fig. 3). Denitrification measured for intact cores was highly variable and statistically only marginally different between treatments (F = 4.75;

Table 3. Soil (0 to 15 cm) properties measured (mean $\pm$ SD) before 20 Nov. 2004 organic amendment.

Management	pH		Bulk density		Organic C		Total C		Total N		POM†		C/N ratio
			—Mg m^{-3}—						—Mg ha^{-1}—				
Organic	7.99	0.13	1.12	0.1	61.5	3.9	82.7	7.09	5.06	0.3	81.7	11.3	12.2
Conventional	7.94	0.06	1.07	0.1	57.5	2.71	81.7	7.78	4.72	0.2	70.7	11.2	12.2
Reference	7.91	0.06	0.76	0.03	51.4	3.76	75.5	10.52	4.29	0.5	–	–	12.1

† POM, particulate organic matter.

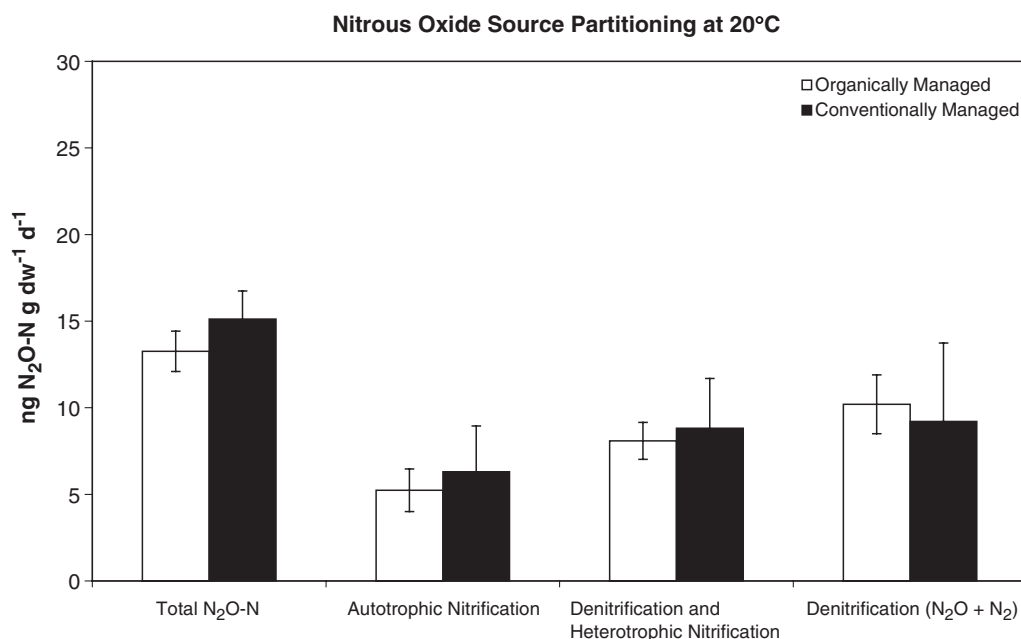**Nitrous Oxide Source Partitioning at 20°C**

Fig. 2. Nitrous oxide flux partitioning for comparison between organically and conventionally managed soils collected in July and at 23% WFPS. Soils were incubated under ambient air, then under 10 Pa C_2H_2, then under 10 K Pa C_2H_2 at field moisture and temperature to determine sources of rates of N_2O emissions and N produced during denitrification. Mean ($n = 10$) $\pm$ SEM are shown for each treatment and incubation.

$p < 0.1$), with a tendency for greater rates in T2O, compared with T2C (Fig. 3). There was no evidence of denitrification for the uncultivated reference soil. Water-filled pore space was similar for both T2O and T2C field soils, with an average %WFPS of 41.4 $\pm$ 5.0 and 39.1 $\pm$ 5.8, respectively. While denitrification occurred in both treatments, greater N_2O emissions following incubation with 10 K Pa C_2H_2 suggest complete reduction to N_2 was favored in the manure-amended soil, compared with unamended soil.

The repeated measures analysis of variance for winter field fluxes indicated N_2O flux was positively affected by organic amendment ($F = 14.95$; $p < 0.01$) and soil temperature ($F = 32.85$; $p < 0.001$) was a significant covariant (Fig. 4). Soil temperatures below freezing were recorded at the study site from 22 November to 15 March, and sub-zero soil temperatures (6 to 8 cm) were also recorded at the weather station during this time (Fig. 5). N_2O emissions were greater in winter than in summer for both treatments (Fig. 4). Average

Nitrous Oxide Emissions and Denitrification from Intact Cores at - 2.5°C
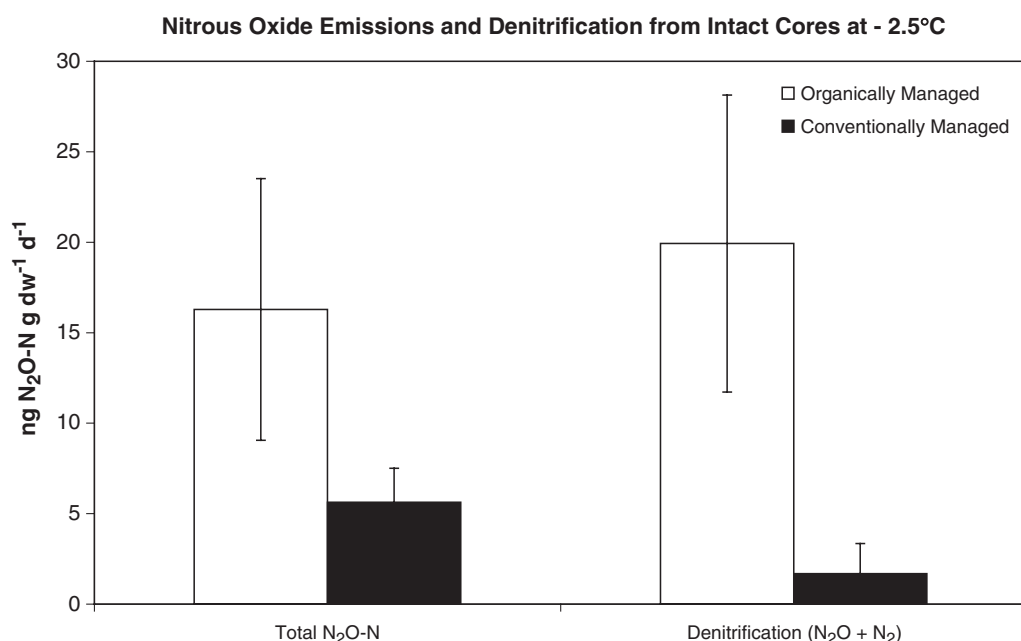

Fig. 3. Nitrous oxide flux and denitrification for intact core incubations at field moisture (40% WFPS) and temperature (−2.5°C) 17 d following T2O manure amendment. Soil cores were incubated under ambient air, then under 10 K Pa C_2H_2 to determine rates of N_2O emissions and N produced during denitrification. Mean ($n = 5$) $\pm$ SEM are shown for each treatment and incubation.

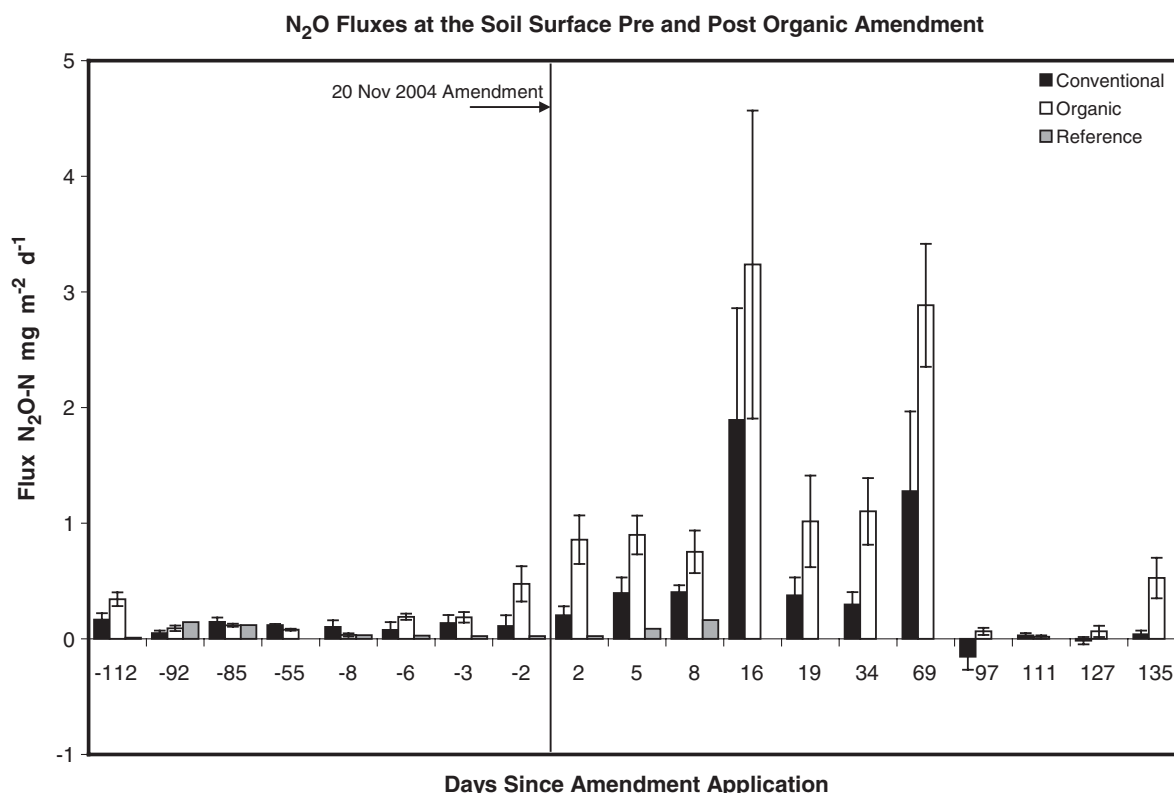**N$_2$O Fluxes at the Soil Surface Pre and Post Organic Amendment**

Fig. 4. Nitrous oxide fluxes (mean ± SEM) measured at the soil-atmosphere interface between 4 Aug. 2004 and 8 Apr. 2005 with respect to number of days following manure amendment application for organic (T2O), conventional (T2C), and reference sites. Soybeans were in both fields between days −112 and −55. Fields were fallowed between days −8 and 135.

(± SEM) daily N$_2$O fluxes from 16 November to 8 April were 1.19 ± 0.34 mg N$_2$O–N m^{-2} d^{-1} for T2O and 0.47 ± 0.37 mg N$_2$O–N m^{-2} d^{-1} for T2C. Highest emissions measured for a single site within treatment was found on 10 December (7.49 mg N$_2$O–N m^{-2} d^{-1} for T2O and 5.75 mg N$_2$O–N m^{-2} d^{-1} for T2C), when soil temperature was −6.1°C. On this date, average N$_2$O emissions were also greatest for both T2O (3.24 mg N$_2$O–N m^{-2} d^{-1} ± 2.98) and T2C (1.89 mg N$_2$O–N m^{-2} d^{-1} ± 2.16).

For comparison with core fluxes measured in the laboratory on 7 December, field fluxes collected when both air and soil temperatures were near −2.5°C were recorded on 14 December. On this date, average soil flux for T2O was 1.10 mg N$_2$O–N m^{-2} d^{-1} ± 0.88 and average soil flux for T2C was 0.38 mg N$_2$O–N m^{-2} d^{-1} ± 0.35 (Fig. 3), which are comparable to core incubations performed the previous week. Although highest N$_2$O emissions were measured when soil temperatures were below freezing, these data represent soil fluxes in late summer and winter only. Soil disturbances associated with tillage and synthetic fertilizers commonly invoke a flush of soil gas emissions (Wagner-Riddle et al., 1997; Venterea et al., 2005) not measured in this study.

Time-integrated winter N$_2$O fluxes were significantly different between conventional and organic soils (F = 36.24; $p < 0.05$). Previous agricultural field studies suggest effects of manure on N$_2$O flux persist 42 to 56 d when applied during the growing season and emissions amount to ~1% of the manure N applied (Lessard et al., 1996; Petersen, 1999). Here, integrated cumulative win-

ter flux measured for the first 35 d since fertilization totaled 0.49 kg ha^{-1}, or 0.7% of the manure N applied. Integrated, postamendment fluxes measured between 22 November and 8 April averaged 1.63 kg ha^{-1} for T2O and 0.64 kg ha^{-1} for T2C. By taking the difference between treatments as the effect of manure on N$_2$O–N emissions, 1.3% of the manure N applied was emitted as N$_2$O–N, which is slightly higher than the 0.9% global mean for fertilizer-induced N$_2$O emissions (Bouwman et al., 2002). Data reported here represents only one winter in the RRV and one type of manure (pelletized, dehydrated). Further study under variable conditions and with alternative amendments is needed to constrain estimates of N$_2$O–N emissions during winter in RRV agricultural soils.

Previous studies confirm increased biological N$_2$O production and denitrification enzyme activity for soils exposed to freeze–thaw cycles (Christensen and Tiedje, 1990; Rover et al., 1998; Jacinthe et al., 2002), for diffusion of trace gases through snowpack (Sommerfeld et al., 1993), and for soil slurries incubated below zero in the laboratory (Dorland and Beauchamp, 1991). In this study, N$_2$O emissions measured in the field may have originated in soil microsites where temperatures were above freezing. For example, N$_2$O produced below the frost zone may have diffused upward and/or N$_2$O may have been produced near the surface on those days when there was sufficient radiation to raise surface temperatures above freezing. However, for laboratory incubations, microbial denitrification occurred below 0°C. Both amended and
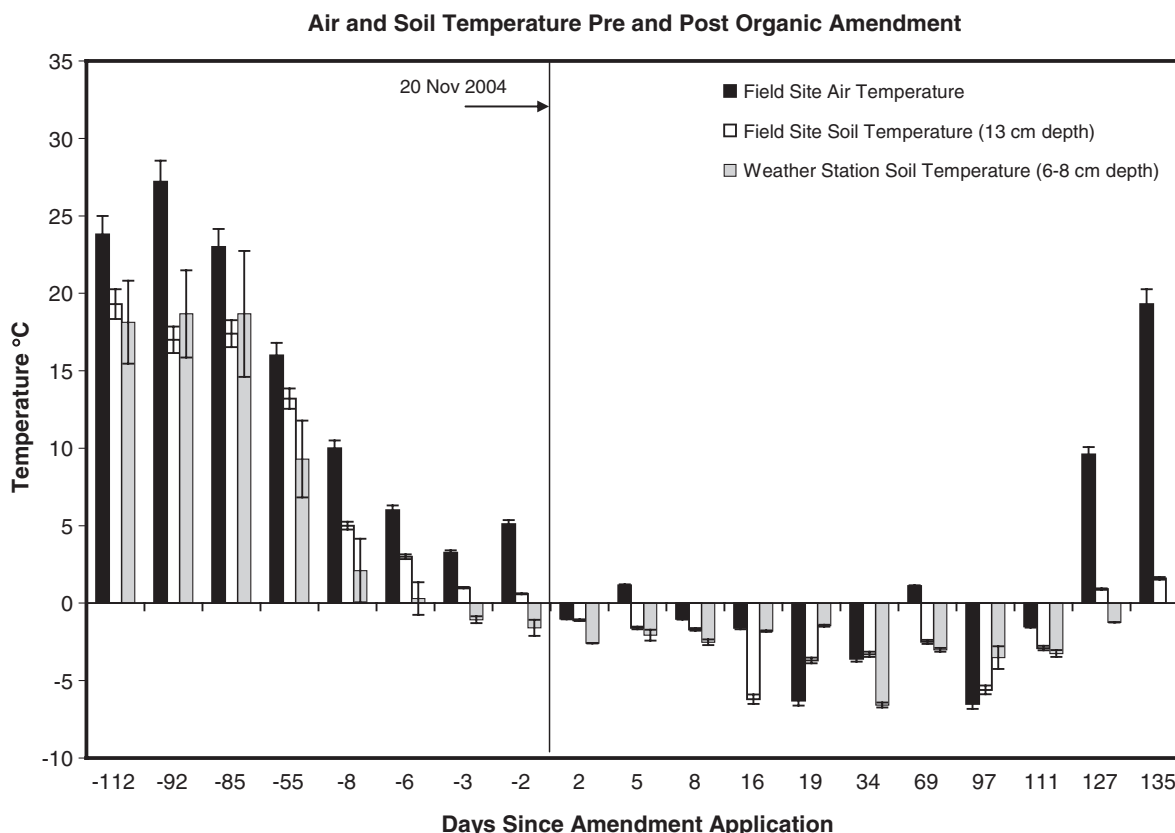
Air and Soil Temperature Pre and Post Organic Amendment

Fig. 5. Mean ($\pm$ SD) for air and soil temperature collected manually at the field site for each field during flux sampling and soil temperature collected continuously at the weather station located north of the field site (see Fig. 1).

unamended soils emitted N_2O, with at least a portion of N_2O flux from denitrification. Trapped gas in soil pore spaces may explain N_2O emissions to some extent (Huttunen et al., 2003), but it does not account for differences between treatments. For organic soils amended with dehydrated manure, denitrification rates were likely stimulated by mineralization of readily available C and N provided by the substrate (Ginting et al., 2003). Microbial ammonification and denitrification of supercooled soils have been reported for slurries in the laboratory, with temperature thresholds strongly influenced by organic substrate supply (Dorland and Beauchamp, 1991). In this study, soil C was relatively high (Table 2), and denitrification occurred for both T2O and T2C soils. Mineralization of the C- and N-rich manure likely began shortly after incorporation into soils, despite subsequent low soil temperatures (Dorland and Beauchamp, 1991; Mikan et al., 2002). The resultant increase in substrate supply from mineralization to the microbial heterotroph population should have enhanced denitrification among microbial communities well-adapted to soil temperatures below freezing, compared with unamended soil.

CONCLUSIONS

Late autumn manure application influenced N_2O fluxes at sub-zero soil temperatures. Denitrification contributed to winter N_2O fluxes, even at $-2.5°C$. Although an additional 1 kg ha^{-1} of N_2O–N was emitted from the surface of manure-amended soils, it remains to be shown if ap-

plication of dehydrated manure in the spring would result in lower N_2O emissions. More importantly, the question of how well manure N mineralization rates track rapid plant growth is not entirely certain (Paul and Beauchamp, 1994), which would support winter manure application recommendations. Clearly identification of interactions between timing of organic amendment application, gaseous N emissions, and mineralization rates are critical for constructing strategies for optimum N conservation in organic production agriculture ecosystems.

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